RECENT INVESTIGATIONS ON THE PROTOPLASM OF PLANT CELLS AND ITS COLLOIDAL PROPERTIES

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I have the honor of publicly congratulating the Representatives of the Missouri Botanical Garden upon the Twenty-fifth Anniversary of Henry Shaw's magnificent foundation,—the unique memorial of a magnanimous citizen of this great metropolis.

I shall endeavor to show to the members of this splendid assembly how plant physiologists at present attempt to reach a satisfactory understanding of the wonderful mechanism which in never-ceasing variation is unfolded to us in myriads of phenomena characteristic of nutrition, reproduction, adaptation, growth, and stimulation, in the lower as well as in the higher plant organisms.

Wherever science is following these various processes to their mysteriously hidden roots, the physiologist has to face the complex problems associated with the living content, the so-called protoplasm of the plant cell. Without this singular matter plant cells are mere dead bodies able neither to grow, to take up food, nor to assimilate their nutriment.

It was not until 1841 that Hugo von Mohl, the well-known botanist of Tübingen, discovered the important fact that all phenomena in cell life are strictly confined to the thin layer of slimy material which clothes the inside of each growing and living plant cell. He stated that this protoplasmic slime was stained deeply yellow by means of iodine, and he expressed the opinion that protein substances in particular were the constituents of this living material, from which all other parts and organs of the cell were believed to take their origin.

We shall not be surprised to learn that biologists felt inclined to suppose that the protoplasm might contain some

peculiar and highly complex proteins constituting the living matter in the proper meaning of the word, whose chemical qualities we should have to make responsible for the whole complex of life phenomena. Therefore, it appeared a most attractive problem to subject protoplasm to a thorough chemical investigation. The names of Reinke and Rodewald are connected with this work. These two botanists, in 1880, then in Göttingen, analyzed the protoplasmic mass, the so-called plasmodium, of *Fuligo septica,* a common species of the *Myxomycetes.* The result was that a part, about three-quarters, of the material was recognized to belong to the protein group in the widest sense; while 25 per cent was a mixture of diverse carbohydrates, fatty bodies, organic acids, and inorganic materials. No evidence of the presence of any peculiar protoplasmic substances was found. Reinke, therefore, laid emphasis on the point that protoplasm could not be regarded as a single chemical body of peculiar qualities, but that it should be considered as a mixture of various substances, of which not even one was unknown to the chemists. The consequence of this view was that Reinke inclined to the hypothesis that the peculiarities of protoplasm were not due to its chemical nature but rather to its peculiar structure. The stuff-hypothesis had to be replaced by a structure-theory of protoplasm.

At present, however, we can scarcely accept all conclusions drawn by Reinke from his famous analysis of protoplasm. Reinke thought that all the vital properties of living protoplasm were destroyed when cells were killed, in the same way as the mechanism of a watch is destroyed by grinding it down in a mortar. The chemical substances, however, may remain unchanged while the mechanism is forever destroyed. The first experiments which proved that Reinke's simile is not quite an exact one were obtained from studies on the various enzyme effects which continue in a mass of finely comminuted tissue. Among those effects we know a series of processes which undoubtedly belong to the complex of vital metabolism, —as, for example, to those of respiration and digestion. And these effects may be followed for weeks and for months after

trituration of the cells, if precaution is taken to prevent change in the material by bacterial action. But the essential difference between such autodigestion and the life-process consists in the fact that the first is not ruled by the laws of correlation and regulation, which are so peculiar to life processes. Nevertheless, we cannot say that the whole of the life-mechanism is destroyed by grinding down living organs. At least a part of it cannot immediately be transformed by this type of disintegration. From this we may draw the conclusion that there are certain chemical substances present in protoplasm which are responsible for certain activities of the living tissue. Such substances are the enzymes, which are entirely unknown in inanimate nature, and absolutely distinctive of cell protoplasm. Further, we cannot suppress some scruple that in Reinke's analysis there were examined not the original protein-bodies of protoplasm, but only substances artificially produced during the treatment of the original material.

Our chief objection against the "Engine-Theory" of protoplasm is that no mechanism has hitherto been known which may be destroyed by heat as easily as is protoplasm, whilst on the other hand one cannot immediately and entirely destroy it merely by pounding to an impalpable pulp. Besides this, recent investigations on the proteids of animal organs—in which great care was taken to dry the pulp quickly at a temperature as low as possible—have shown that there really exist highly compounded protein bodies of hitherto unknown constitution which have to be considered as real constituents of protoplasm.

Can such discoveries in some way explain the vital properties of the cell? It seems as if we may not understand the wonderfully accurate working-together of all organs in cells without supposing trans-microscopical structural qualities; but we need not assume any mysterious new forces or structures. Most of the well-known characteristics of protoplasm can be understood by considering further the colloidal state of the constituents of the cells.

The first naturalist who turned his attention to the great importance of colloidal substances in cells was Bütschli, the zoölogist of Heidelberg. A great number of his admirable papers deals with the microscopical features of cell plasma, which he described as a framework of jelly-like substances containing interstices, or meshes filled with fluid substances. Bütschli emphasized the view that the foam structure described by him is not peculiar to living matter, because a mixture of oil and gelatin solution shows the same microscopical structure which he attributed to protoplasm and to all colloids.

But later on it became more and more probable that such a foam structure in protoplasm indicates nothing more than certain gross features which are by no means identical with the real colloidal structure of plasmatic constituents. Not even in gels, or solid colloids, apparently, is the foam structure a dominant characteristic. Zsigmondy's recent work on gelatinous structure clearly showed that while forming the gel the colloidal particles, which are distinctly visible in the ultramicroscope, do not arrange themselves in a network, but settle quite irregularly; so that we cannot assume that meshes are formed in the precipitation of colloids. On the other hand, biologists of rank, as Lepeschkin, after a careful study of the microscopical structure and the physical properties of protoplasm, have arrived at the conclusion that we should not regard it as a foamy mass, or jelly-like substance, but rather as a liquid colloid with the characteristics of protein sols of certain higher concentrations. We can easily confirm the observation that protoplasm, examined by means of the highest power of the microscope, often appears merely as a homogeneous liquid, or transparent mass, sometimes moderately turbid from the presence of small distinct drops or corpuscules which are collectively known under the name of "microsomata." Even though we do not accept Bütschli's idea with respect to specific structure, we fully share his more general point of view that living protoplasm owes its peculiar activities to colloidal qualities. And this represents our attitude to-day towards protoplasmic investigation.

The chemistry of colloids is not a descriptive science. To the utmost extent it has to use experimental physical methods. So we cannot advance in knowledge of protoplasm by mere microscopical observation, but mainly by experimental investigation.

A long time even before colloidal chemistry became dominant as the basis for the physiology of protoplasm, a memorable epoch in plant physiology had opened, developing from the ingenious work of Pfeffer and De Vries on the osmotic properties of living cells. These investigations unveiled the fundamental fact that living protoplasm alone is in possession of those peculiar properties of permeability which are responsible for the whole complex of nutrition. Dead protoplasm behaves quite differently. Since, however, differences in respect of the penetration of different solutions can be detected to a certain extent in colloidal membranes, it became probable that the so-called semipermeability of living protoplasm is a colloidal phenomenon, due to the constituent colloids in living protoplasm; whilst after the death of the cells the coagulation of these colloids completely changes the peculiar permeability of the protoplasmic layer.

It was, however, Ernest Overton, in 1899, then at Zurich, who acquired the merit of placing colloidal chemistry in fundamental relation to the phenomena of diosmosis in living cells. The well-known theory of Overton consists in the hypothesis that fatty substances play an important rôle as constituent elements in the protoplasmic matrix. It is due to such substances, generally comprised under "lipoid bodies," that living cells show quite distinctive diosmotic qualities. Overton's hypothesis is founded upon the fact that only those substances which readily dissolve in fatty oils are easily diffusible in living cells; whilst all substances which are insoluble in oily media, as sugar or mineral salts, easily produce plasmolysis, because they penetrate into cells only very slowly.

The leading physical idea in this theory was the so-called "Partition-Rule" of Berthelot and Jungfleisch. This law states the fact that there exists a constant relation between the quantities of a certain solute dissolved in two immiscible

solvents. Overton considered the endosmosis of dissolved substances into living cells as merely a question of solubility. It is known how fertile this idea has proved in physiology, particularly in the phenomenon of narcosis, where it is still the leading hypothesis in animal physiology.

But recently experimental work, including my own, has shown that it is scarcely quite correct to consider the endosmosis of solutions into living cells as a typical solution phenomenon. According to Loewe even the partition of methylene-blue or of chloroform between oil and water cannot readily be explained by means of the principle of Henry and Berthelot. Rather, the oily solution of such substances is not a true solution, but only a colloidal solution; so it is not ruled by the laws of osmotic pressure, but by the laws of adsorption.

A striking fact was discovered by Traube and by myself in studying the effects of alcohols and other capillary-active substances on living cells. Their injurious action clearly and exclusively depends upon the relative capillary activity. Every one of these substances kills the cells at a concentration corresponding exactly to a certain value of surface tension. The main importance of this observation consists in the evidence that in narcotic effects capillary phenomena must be a dominant factor. This cannot be interpreted by the supposition that the entrance of narcotics into cells is due to true solution phenomena. The observed capillary effects distinctly show that the factor of real moment is to be found in alterations of contact-surface; but such surface-phenomena are met with only in colloids and in their adsorption.

A prominent feature of our experiments involves the fact that cells of higher plants are constantly killed by concentrations of narcotics such that the capillary activity reaches about two-thirds of the surface tension of pure water in contact with air. It is remarkable that saturated and neutral emulsions of triolein or other typical fats always show approximately the same surface tension value. This result I tried to explain by means of the hypothesis enunciated in the following sentences: Alcohol and other narcotics are taken up by ad-

sorption into living protoplasm. According to the theorem of Willard Gibbs the surface in liquid systems which consist of different fluids and contain some capillary-active substances is always occupied by those substances which show the greatest reduction in the surface tension of the medium. If subsequently another substance with greater capillary activity is added to the system it displaces all other substances from the surface. Narcotics may displace certain plasmic substances in an analogous way, provided that the surface tension of the concentration applied is just a little lower than the surface tension of the plasmatic substances referred to. The fact that the fatal narcotic pressure value coincides with the maximum surface tension in fat emulsions may be explained by the hypothesis that fatal effects of alcohols on living cells consist in destroying the emulsion structure of protoplasm, by displacing some fatty substances. So our experiments to a certain extent uphold the view that the surface layer of protoplasm really contains fat, and thus far is in accordance with Overton's hypothesis.

In the course of time the lipoid-theory of Overton has met with sharp criticism. Among other renowned physiologists, Ruhland strongly denied the presence of fatty bodies in the plasmatic membrane of plant cells. On the other hand, we are aware that animal physiologists, such as Fühner, Höber, and Vernon still firmly adhere to the old lipoid-theory. However, since according to Overton sugars and mineral nutrient salts are believed to penetrate only poorly into the living cell, it is obvious that Overton's hypothesis stands in direct contrast to the common experiences in respect to plant nutrition. The substances referred to are materials which the cells have to take up as among their most important nutrients. Nevertheless, there have been developed some supplementary theories which permit us to lessen the difficulties of the lipoid-theory, for example, that of Nathansohn, according to which the lipoid membrane of protoplasm is not a continuous film of fat, but a kind of mosaic of fat and protein which is able to permit the penetration of both fat-soluble substances and mineral salts.

Ruhland's experiments especially were not at all favorable to the lipoid-hypothesis. They show decidedly the error of the opinion that only those aniline dyes penetrate into living cells which are soluble in oil. Many aniline dyes have been found which are easily taken up by cell protoplasm in spite of their insolubility in fat, while other coloring matters which easily dissolve in fat do not penetrate at all through the living plasmatic layer. Ruhland, as well as Küster, drew from such experiments the convincing conclusion that substances readily soluble in lipoids may not always be readily taken up by the living cells. But in other respects it seems as if Ruhland had gone too far when he denied that protoplasm possesses any fat content. He emphasized that he never could detect any microscopical trace of plasmatic substances which may be stained by means of such aniline dyes as are readily stored by fat.

Since our own experiments seem to be in some accord with the view that fatty matter really is present in protoplasm, I wanted to compare some chemical systems which are entirely free from fat with protoplasm in respect to its behavior toward alcohols. It could be taken as a proof of the view that protoplasm does not contain fatty bodies, if there were noticed no difference between the effects of alcohols on the physical properties of such systems and on protoplasm. The investigations of Mr. Geo. H. Chapman in our laboratory were begun in order to examine the influence of different narcotics on enzymes. Surprisingly, the results were opposed to the above-mentioned view of similar action with respect to these systems. This work clearly showed that the capillarity-rule which is so distinctive of the effects of narcotics on living protoplasm does not apply to the effects of narcotics on enzymes. While the deleterious influence of methyl, ethyl, and propyl alcohol gradually increases with the molecular weight of these homologous substances, the higher members such as butyl and amyl alcohol act considerably less on enzymes, and both heptyl and octyl alcohol have practically no weakening influence on these ferments. In respect to their coagulation by diluted alcohol protein solutions show relations corresponding to

those just discussed. In consequence of this result we can hardly explain the effects of narcotics on protoplasm by the view that only plasmatic protein bodies are influenced by such toxic agents. Besides this, for the coagulation of protein bodies there is required not less than five mols of ethyl alcohol while a little more than two mols is sufficient to kill living protoplasm. Therefore, some other substances in protoplasm besides the protein bodies must be affected by the alcohols, and these substances must differ from the latter in their physical properties. So it seems that the view according to which the plasmatic membrane is constructed exclusively of hydrocolloids, viz., proteins, as Ruhland believes, cannot be considered to be quite satisfactory. Our attention must be directed anew to the possibility that some lipoids play the part of important constituents of the protoplasmic membrane.

On the other hand, I have to state that several lines of experimental work have led us to the conclusion that the endosmose of solutions into living cells never does take place by way of plasma lipoids, but only through hydrocolloidal constituents of the cell plasma. The work of Mr. Krehan, which dealt with the influence of highly diluted hydrocyanic acid on plant cells, distinctly showed that in the presence of this agent the permeability of cells to certain salts, such as sulphates, and to sugar, is raised, so that the threshold of plasmolysis for these substances is raised. When the effects of different salts on plasmolysis were compared it became manifest that just those salts causing the greatest rise of the plasmolytic limit, are those which were strongly adsorbed, and which display a most marked effect on the precipitation or coagulation of albumen. Such salts are sulphates, citrates, tartrates—by their anionic effects, and the salts of ammonium, calcium, and magnesium—by their cationic effects. These phenomena are only to be understood upon the supposition that hydrocolloids are the media through which different substances must pass when taken up by the living cell plasma. There has been discovered not the faintest indication that

lipocolloids can play an important part in endosmose, as Overton originally suggested.

If there really are plasmatic lipoids present, they probably have no significance as the path of nutrient substances into cells. But, on the other hand, lipoids certainly participate in narcotic effects, because the more soluble is this narcotic in fat the more of the narcotic substance is stored by the plasmatic substances. Consequently, the higher members of the series of alcohols are more injurious for cells than the lower, because the lipoid constituents of protoplasm become saturated with the narcotic and can discharge these narcotics only slowly. So the protoplasm succumbs to the influence of the narcotic agent. On this point I share the opinion of Böeseken and Waterman.

The capillarity-rule can scarcely be explained otherwise than by the hypothesis that lipoids are present in the surface layer of protoplasm. So we are forced to continue our work as an exploration designed to determine if lipocolloids are present in protoplasm. A plan was devised and a decision was sought in the following manner: Emulsions of pure triolein or of olive-oil were prepared which had about the same surface tension value as have solutions injurious to protoplasm. To a series of samples arranged from such a fat emulsion alcohol in gradually increasing amount was added. The question now was whether there were effects produced on the emulsion in some way comparable to the action of alcohol on cells. Cell plasma contains also protein bodies and mineral salts. So our model of emulsion had to be compounded by adding a solution of mineral salts, as a physiologically balanced mixture, and by adding also albumen solution. The mineral salts were added as in the Van't Hoff mixture in 0.1 molar concentration. An alkali is indispensable, so that 0.1 mol of sodium carbonate was used in order to produce a fine and stable emulsion upon shaking the mixture with oil. The results were in brief the following: When a fat emulsion from olive oil was prepared by mixing only oil, water, and sodium carbonate, the decomposing effect of alcohol on the emulsion was noticed at a concentration of 3 mols, i. e., about 15 per

cent. When concentrations higher than this were used then the emulsion, examined capillarimetrically, did not differ from a mixture of pure alcohol and water of the same concentration (but without oil). Then we added to the emulsion Van't Hoff's solution 0.1 mol instead of water. The decomposition of the emulsion by ethyl alcohol was now observed at 2 mols, i. e., about 10–11 per cent. This is just the concentration of alcohol which kills cells of the higher plants. The addition of sodium chloride 0.1 mol instead of Van't Hoff's liquid showed the critical concentration of alcohol to be 3 mols, about the same concentration as in the absence of mineral salts. On the other hand, the addition of magnesium chloride induced the fatal effect of alcohol at 1 mol, much lower than in living cells. Magnesium sulphate showed the same effect as magnesium chloride, and the sulphate of sodium the same as the chloride. Therefore, it does not seem probable that the differing solubility in alcohol is responsible for the various effects of the salts. One may endeavor to explain these phenomena in the following way: Emulsions are only stable when the droplets of the emulsified fat remain suspended in a soap solution of approximate concentration. Substances which alter the limiting surface between the soap solution and the suspended oil must prove fatal as soon as their capillary activity surpasses the capillary effect of the soap solution. Bivalent cations, such as Mg and Ca, which form insoluble salts with fatty acids, lower the concentration of soap, so that alcohol must exhibit a decomposing action on the emulsion, even in lower concentrations.

From such experiments it seems as if the critical concentration of alcohol for living cells would not be so sharply determined by proteins contained in protoplasm as by the mineral salt and the lipoid constituents of the protoplasm. Since we suppose that the various mineral salts in protoplasm are present in about the same concentration as they are found in sea water, or as they are mixed together in Van't Hoff's solution, we have to face the question whether the destructive effect of alcohol on living cell plasma consists in some decomposition of colloidal fat emulsoids in protoplasm.

That protein bodies are not primarily affected by alcohol and other narcotics seems to be sufficiently proved by the fact that ethyl alcohol coagulates protein solution at a concentration not lower than 5 mols, and that while the higher alcohols show fatal effects on living cells, they do not produce any protein coagulation.

So we are brought, I think, by several facts to the conclusion that living protoplasm must be considered as a colloidal emulsion of lipoids in hydrocolloidal media, the latter containing proteins and mineral salts. For the endosmotic passage of dissolved substances the fatty constituents of protoplasm have no significance. The narcosis, however, and the deleterious effects of alcohols clearly show how lipoids, more than the protein constituents of the surface layer of protoplasm, participate in such phenomena. The more we advance in the disclosure of the details regarding colloidal mixtures and structures in living protoplasm, the more indispensable it is to be reserved when applying the new results to the various problems to which an approach is so tempting to the physiologist.

Many may feel inclined to be disappointed when they observe how much time and mental energy are needed to study only so small a question as that about the presence of fat in protoplasm. But now after some years' work on this subject it may be seen how important a part is to be attributed even to the combination of mineral salts contained in the plasma colloids. And so we may hope that in the progress of research new and unexpected paths may become visible and open to the indefatigable investigator. Further, we shall not be discouraged if when after long and patient work some results and ideas are won which subsequently are proved untenable. We are all common soldiers in the great battle for truth in science, and we know that few will attain the happiness of planting the flag of victory upon the battlements of the conquered fortress.